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Canine distemper, a re-emerging morbillivirus with complex neuropathogenic mechanisms

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Abstract

Paramyxoviruses are responsible for a wide variety of diseases both in humans and in animals. Common to many paramyxoviruses is the fact that they can cause neurological symptoms in their final host. Newly discovered paramyxoviruses, such as the Hendra and Nipah viruses, show the same pattern of pathogenesis as that of the paramyxoviruses already known. Canine distemper virus (CDV) is a well-studied member of the genus *Morbillivirus*. Study of the neuropathogenesis of CDV might give insight into disease mechanisms and suggest approaches for the prevention of other recently discovered paramyxovirus infections.

Keywords: canine distemper, morbillivirus, dogs, henipah, neuropathology

Introduction

Members of the family Paramyxoviridae are responsible for a variety of important diseases affecting livestock (e.g. rinderpest, Newcastle disease), pet animals (e.g. canine distemper) and man (e.g. measles) (Table 1). The most significant economic losses in livestock are caused by rinderpest virus and Newcastle disease virus. Canine distemper is the most important viral disease in dogs, with high morbidity and mortality in unvaccinated populations worldwide. In humans, measles continues to be a significant cause of infant mortality in developing countries. Also, in industrialized countries, outbreaks of importance are occasionally observed in children, probably as a result of inadequate vaccination programs.

During the past two decades, several novel paramyxoviruses have emerged (such as Hendra, Nipah and phocine distemper virus) and several previously described paramyxoviruses [such as canine distemper virus (CDV)] have crossed the species barrier. Many of these newer and older members of the family Paramyxoviridae can cause neurological symptoms because of invasion of virus and/or immune cells into the central nervous system (CNS). The detailed studies of the neuropathogenesis of CDV that have been carried out might therefore give some insight into similar disease patterns among the emerging paramyxoviruses. This review provides a brief summary of our knowledge of important emerging paramyxoviruses and of the pathogenesis of CDV in dogs.

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Henipah, a new genus in the family Paramyxoviridae

Recently, a new group of paramyxoviruses closely related but distinct from the genus *Morbillivirus* has been discovered. These viruses, which were first identified in Australia (Hendra) and Malaysia (Nipah), cause subclinical infection in fruit bats, and have caused mortality in horses, pigs and humans. Both viruses were initially classified as *Morbillivirus*-like, but will now be assigned to a new genus, *Henipah*, within the paramyxovirus family (Mayo, 2002) (Tables 1 and 2).

Table 1. Examples of the family Paramyxoviridae

Genus	Virus	Host	Re-emerging and/or emerging	Vaccination[2]
Respirovirus	Parainfluenza 1–3	Mammals	No	Yes
	Paramyxovirus	Pig	Yes	Yes
Morbillivirus	Measles	Human	Yes	Yes[3]
	Canine distemper	Carnivores, seal	Yes	Yes
	Phocid distemper[1]	Seal	Yes	No
	Dolphin morbillivirus	Dolphin	Yes	No
	Rinderpest	Cattle	No	Yes
	Peste des petites ruminantes	Sheep, goat	No	Yes
Rubellavirus	Mumps	Human	No	Yes
	Newcastle disease	Fowl	No	Yes
Pneumovirus	Bovine respiratory syncytial	Cattle	No	Yes
Metapneumovirus	Turkey rhinotracheitis	Turkey	Yes	Yes
Henipah	Hendra	Horse, human[5]	Yes	No
	Nipah	Pig, human[5]	Yes	No[4]
Unassigned	Menangle	Pig, human[5]	Yes	No

[1]Also called phocine distemper virus; [2]common vaccination practice; [3]vaccination coverage in certain areas, population groups rather poor; [4]experimental vaccine (T. McKenna, personal communication), [5]fruit bat reservoir, modified after Wang and Eaton, 2001.

In September 1994, a sudden outbreak due to a previously unknown agent caused the deaths of 13 horses and a human due to a hyperacute respiratory disease with mild neurological symptoms. Subsequently, another outbreak was recorded in a single horse in Cairns (Australia) in 1999 (for review see Barclay and Paton, 2000; Hooper and Williamson, 2000; Mackenzie *et al.*, 2001; Wang and Eaton, 2001). The virus is not highly contagious since transmission from horses to other horses and to humans occurs only after very close contact with an infected individual. Serological studies have shown that other species, such as dogs, cats and livestock species, have not been infected by the virus so far. The causative agent was initially called 'equine morbillivirus', but subsequent genetic analyses showed that its most appropriate classification is as the prototype member of a new genus within the family Paramyxoviridae (Murray *et al.*, 1995, 1998; Rogers *et al.*, 1996; O'Sullivan *et al.*, 1997; Chua *et al.*, 2000; Harcourt *et al.*, 2000; Wang *et al.*, 2000; Mayo 2002).

Although it had been presumed that Nipah virus had caused a low level of mortality in pigs in Malaysia since 1996, the virus remained unknown until September 1998, when a major outbreak in humans and pigs resulted in over 265 infected humans, 105 of whom died of encephalitis, and the slaughter of over 1.2 million pigs since then. In contrast to Hendra virus, Nipah virus was found to be contagious and the authorities were forced to choose culling as the only option to control the spread of the virus (Chua *et al.*, 1999, 2000, 2002; Mackenzie *et al.*, 2001). Unlike the respiratory disease caused by Hendra virus in horses, Nipah virus infection in pigs led to subclinical infection with mild respiratory symptoms and occasional CNS involvement (Table 2). In humans, Nipah virus infected the CNS and caused encephalitis and death (Lam *et al.*, 2002). In contrast to Hendra virus, Nipah virus appears to be able to infect other animals, such as dogs, rats, and even a variety of avian species. The introduction of intensive pig farming in areas where the fruit bat is present as a vector was probably the direct cause of the appearance of Nipah virus in pigs. Recent studies even suggest that Nipah virus is more widespread in Southeast Asia than previously documented (Olson *et al.*, 2002).

The detailed molecular characteristics of both Hendra and Nipah virus have been published (for review see Wang and Eaton, 2001). In summary, Hendra and Nipah viruses resemble respiroviruses and morbilliviruses more closely than rubellaviruses. However, they have features that make them unique and distinct and therefore justify classification as a new genus within the family Paramyxoviridae, *Henipah* (Wang and Eaton, 2001). Since Hendra and Nipah are zoonotic viruses, it needs to be pointed out that work with these viruses must be carried out strictly in approved biosafety level 4 laboratories.

In addition, there are several other viruses not yet assigned to the paramyxovirus family. Menangle virus was isolated in 1997 from piglets in Australia, and two humans seroconverted after having close contact with the infected animals. It is likely, with increasing disease awareness and surveillance, that new viruses of the family Paramyxoviridae will be found in other species.

Table 2. Diseases caused by members of the *Morbillivirus* and *Henipah* genera discussed in this review

Virus	Host	Zoonotic agent	Disease	CNS involved[1]	Geography	Outbreaks
Measles[2]	Human, monkey		Generalized	Rare	Worldwide	Year round
Canine distemper	Wide range of canids	Possible	Generalized	Yes	worldwide	Year round
	Lion		Generalized	Yes	Africa (Serengeti)	1994
	Siberian/Baikal seal		Generalized	Yes	Lake Baikal	1987, 1988
	Caspian seal		Generalized	Yes	Caspian Sea	1997, 2000
Phocine distemper	Harbor/gray seal		Respiratory	Yes	NE Europe, USA	1988, 2002
Dolphin morbillivirus	Dolphin		Respiratory	Yes	Mediterranean, USA	1987, 1990
Porpoise morbillivirus	Porpoise		Respiratory	Yes	Ireland, Gulf of Mexico	1988, 1994
Hendra	Horse	Yes	Respiratory	Rare	Australia	1994, 1999
Nipah	Pig, others?	Yes	Respiratory	Rare[3]	Malaysia	1996[4], 1998

[1]In the principal host; [2]Measles virus can be regarded as the prototype member of the *Morbillivirus* genus; [3]in humans Nipah virus predominately caused encephalitis; [4]Nipah virus probably responsible for low mortality in pigs in Malaysia in 1996.

Morbilliviruses of carnivores and aquatic mammals

CDV is the only member of the genus *Morbillivirus*, which in the past has been associated with disease in a wide range of carnivores. In the past two decades, however, CDV has crossed the species barrier on several occasions and has caused significant problems after its introduction into a new species. Many aspects of CDV, including its molecular biology, diagnosis, host range and epidemiology, have been reviewed in earlier publications (Vandevelde and Zurbriggen, 1995; Stettler *et al.*, 1997; Barrett, 1999; Cherpillod *et al.*, 1999; Leisewitz *et al.*, 2001; Deem *et al.*, 2000) and will not be discussed here.

Although live attenuated vaccines have been used successfully for many years to control morbillivirus diseases, canine distemper continues to be a problem, outbreaks occurring in domestic dogs and farmed mink populations. The current vaccines on the market have some severe drawbacks. It was suggested earlier that the insufficient protection provided by the vaccine strains might be the consequence of changes occurring in the vaccine virus after cell culture passages (Stettler and Zurbriggen, 1995; Bolt *et al.*, 1997; Iwatsuki *et al.*, 1997). Other side-effects of vaccination are (i) the potential induction of immunosuppression, (ii) CNS complications (Hartley, 1974; Cornwell *et al.*, 1988), (iii) mortality in species other than carnivores (Bush *et al.*, 1976; Carpenter *et al.*, 1976; Barrett, 1999), and (iv) occasional insufficient protection (Johnson *et al.*, 1995; Gemma *et al.*, 1996; Ek-Kommonen *et al.*, 1997).

Besides the known susceptible species in the order Carnivora, CDV has found many other hosts in past years, and can therefore be classified as an emerging virus in these new target species. For example, CDV was isolated from the African wild dog (van de Bildt *et al.*, 2002), the Caspian seal (Kennedy *et al.*, 2000) and the Baikal seal (Osterhaus *et al.*, 1989) (Table 2). Seroprevalence to CDV has also been shown in the family Felidae, in particular the Serengeti lion (Morell, 1996; Roelke-Parker *et al.*, 1996; Cleaveland *et al.*, 2000) and domestic cats in Japan and Taiwan (Ikeda *et al.*, 2001). Therefore, alternative vaccines, such as DNA vaccines, and vaccine delivery systems (ISCOMS) for CDV (and other members of the paramyxovirus family) are urgently needed.

In addition, viruses related to CDV have been described recently: the phocine (seal) distemper virus (PDV) and the dolphin (Cetacean) morbillivirus (DMV). PDV was first found in harbor seals in 1988, and led to a mortality of 50% of the total harbor seal population in North-Western Europe (Osterhaus *et al.*, 1988; Osterhaus and Vedder, 1988; Appel *et al.*, 1994; Duignan *et al.*, 1995; Harder *et al.*, 1995; Brown, 1997). For unknown reasons, PDV re-emerged in the seal population in several countries in Northern Europe in June 2002, and killed at least 18 000 seals (Jensen *et al.*, 2002). DMV caused mortality among striped dolphins in the

Mediterranean Sea and the Gulf of Mexico (Table 2). This virus is distantly related to PDV and CDV (Barrett *et al.*, 1993). The epidemiology of these outbreaks remains mostly obscure. However, factors that have been suggested to be responsible for the emergence of these viruses in aquatic populations are (i) transmission due to close contact with distemper-infected dogs and other carnivores and aquatic mammals infected with the above-mentioned viruses, and (ii) a weakened immune system as a result of environmental stress (Barrett, 1999; Osterhaus 2001; Wang and Eaton, 2001).

Canine distemper in carnivores

CDV is generally transmitted as an aerosol infection of the upper respiratory tract. The primary replication of the virus takes place in the lymphoid tissues, leading to severe, long-lasting immunosuppression (Appel, 1969; Krakowka *et al.*, 1980; Krakowka, 1982). T cells are more affected than B cells and $CD4^+$ lymphocytes are rapidly depleted for several weeks, whereas $CD8^+$ cells are less affected and recover relatively rapidly. About 10 days after infection, CDV starts to spread from the sites of primary replication to various epithelial tissues and the CNS. As a result of epithelial infection, a variety of respiratory, intestinal and dermatological signs can occur. The most serious complication is infection of the CNS, leading to a variety of neurological symptoms with poor prognosis (Tipold *et al.*, 1996). Understanding of the mechanisms by which CDV causes damage to the CNS is important in the design of new strategies for therapy.

Neuropathology of nervous canine distemper

While some CDV strain variations and related differences in lesion patterns exist (Summers *et al.*, 1984a), in the vast majority of cases of spontaneous and experimental distemper with so-called demyelinating strains, such as R252 (McCullough *et al.*, 1974) and A75/17 CDV (Summers *et al.*, 1979), the virus causes multifocal lesions in the gray as well as in the white matter of the CNS (Summers *et al.*, 1995). The demyelinating lesions are not only responsible for severe neurological signs but are also thought to be a model for human demyelinating conditions, such as multiple sclerosis (Appel *et al.*, 1981; Dal Canto and Rabinowitz, 1982). It has even been proposed by several groups that, on the basis of epidemiological and molecular studies, CDV could be the cause of multiple sclerosis (Krakowka and Koestner, 1978; Cook *et al.*, 1979, 1980, 1986, 1995; Cook and Dowling, 1980; Hughes *et al.*, 1980; Appel *et al.*, 1981; Madden *et al.*, 1981; Rohowosky *et al.*, 1995; De Keyser *et al.*, 2001; Gilden, 2002). Therefore, the pathogenesis of demyelination in distemper has been investigated closely in recent years. In the following, we will focus on the current knowledge of the pathogenesis of demyelination in canine distemper.

Pathogenesis studies have to consider an *acute* and a *chronic* stage in the development of CDV-induced demyelination. The initial lesions occur around 3 weeks after infection and evolve during a period of massive virus-induced immunosuppression (Vandevelde *et al.*, 1982a). Depending on the degree and speed of immune recovery, animals may either become moribund quickly or recover after developing a mild or subclinical illness. An intermediate group of animals recovers slowly or partially, and tends to develop a chronic or relapsing disease with progression of the demyelinating lesions as a result of immunopathological reactions (Vandevelde *et al.*, 1981, 1982b).

The acute stage of CDV-induced demyelination

The initial myelin lesions develop during a period of severe immunosuppression and are not inflammatory (Vandevelde *et al.*, 1982a) (Fig. 1). Several immunocytochemical studies and recent *in situ* hybridization work in spontaneous and experimental distemper have clearly shown that demyelination coincides with replication of CDV in the glial cells of the white matter (Vandevelde *et al.*, 1985b; Zurbriggen *et al.*, 1993). Spatiotemporal studies leave no doubt that the initial white matter lesions are associated with viral activity and that their development is highly predictable (Summers *et al.*, 1979; Higgins *et al.*, 1982; Vandevelde *et al.*, 1985b) (Fig. 2). The most obvious explanation for demyelination would be infection of oligodendrocytes, the myelin-producing cells.

Therefore, research has focused on evidence of CDV (mRNA, viral antigen) in oligodendrocytes. At the light microscope level, it has been shown that the majority of infected cells are astrocytes (Mutinelli *et al.*, 1989). Most electron microscope studies agree that oligodendroglial infection is very rare in distemper (Wisniewski *et al.*,

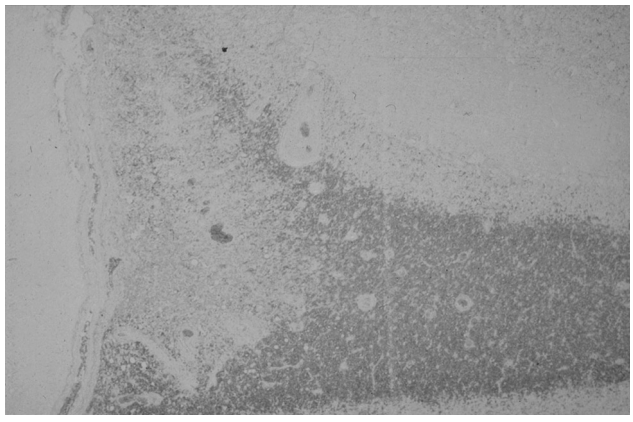

Fig. 1. Acute demyelinated region in the cerebellum after natural canine distemper virus infection. Immunohistochemistry for myelin basic protein showing oligodendrocytes, the myelin-producing cells.

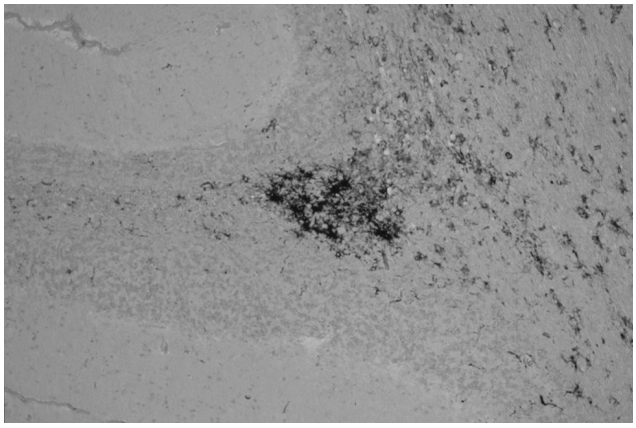

Fig. 2. Canine distemper virus in the white matter of the cerebellum demonstrated by *in situ* hybridization using a probe specific for mRNA coding for the P-protein. Acute phase of the disease.

1972; Raine, 1976; Higgins *et al.*, 1982; Summers and Appel, 1987; Blakemore *et al.*, 1989; Glaus *et al.*, 1990). Very few oligodendrocytes containing CDV antigen were found at the light microscope level. However, approximately 8% of the oligodendrocytes at the edge of lesions contained CDV mRNA (Zurbriggen *et al.*, 1998). This restricted infection of oligodendrocytes was found earlier in dog brain cell cultures (DBCC) (Zurbriggen *et al.*, 1993), in which virulent CDV causes a slowly spreading non-cytolytic infection. CDV proteins or viral nucleocapsids were found only very rarely in oligodendrocytes in culture, in contrast to astrocytes and microglial cells, which easily support CDV infection (Vandevelde *et al.*, 1985a; Zurbriggen *et al.*, 1986, 1987). We conclude from these studies that CDV causes a restricted infection of the oligodendrocytes, which is possibly responsible for the phenomenon of demyelination. Why the production of viral protein does not take place in these cells remains to be elucidated.

Between 20 and 30 days after infection, cultured oligodendrocytes, which grow on top of a layer of astrocytes in mixed DBCC, start to degenerate and disappear, although the supporting culture remains a continuous cell sheet (Zurbriggen *et al.*, 1987). Ultrastructural studies revealed microvacuolation and loss of organelles in such oligodendrocytes (Glaus *et al.*, 1990). The morphological changes are preceded by metabolic dysfunction of these cells, because the activity of cerebroside sulfotransferase—an oligodendrocyte-specific enzyme—decreased markedly soon after infection (Glaus *et al.*, 1990) and myelin transcription is strongly decreased in infected DBCC (Graber *et al.*, 1995). It was shown *in vivo* that CDV infection led to massive down-regulation of myelin gene transcription, with complete loss of *in situ* hybridization for proteolipid protein in oligodendrocytes within demyelinating lesions (Zurbriggen *et al.*, 1998). Morphological changes of oligodendrocytes were also described in the demyelinating lesions *in vivo* using electron microscopy (Summers and Appel, 1987; Blakemore *et al.*, 1989). The fate of the oligodendrocytes remains unclear, and there is no solid evidence that these cells undergo necrosis or apoptosis in CDV infection, either *in vivo* or *in vitro* (Schobesberger *et al.*, 1999).

There is little doubt that a change in these cells is at the root of the demyelinating process, but its mechanism is not yet understood. It is possible that viral transcription taking place in these cells interferes with the specialized functions necessary to maintain myelin membranes. The possibility that these cells are affected as a result of virus-induced changes in other cell types cannot be excluded. However, a series of experiments could not confirm this hypothesis. Supernatants derived from CDV-infected DBCC did not induce oligodendroglial lesions in recipient dog or mouse brain cell cultures (Zurbriggen *et al.*, 1987). We were unable to find evidence of toxic factors, such as tumour necrosis factor α and reactive oxygen radicals, in the supernatants of CDV-infected DBCC (Brügger *et al.*, 1992). Cocultivation of infected DBCC with mouse brain cell cultures, which are refractory to CDV, did not damage the mouse oligodendrocytes (unpublished results, C. Griot and A. Zurbriggen). Likewise, the mouse brain cells (which remained uninfected in these cocultures) did not provide protection for the canine oligodendrocytes (unpublished results, C. Griot and A. Zurbriggen).

In summary, acute CDV infection of the white matter results in metabolic oligodendroglial changes, which lead to demyelination. Whether the change in the oligodendrocytes is the direct result of the restricted CDV infection mentioned above remains to be shown.

The contribution of the immune response to early lesion development is not clear. While an effective antiviral neutralizing immune response is lacking in the acute phase of distemper, anti-CDV IgM antibodies occur within the first 2 weeks of infection (Barben *et al.*, 1999). Despite severe immunosuppression and lack of perivascular cuffing, numerous $CD8^+$ cells are found in acute demyelinating lesions and are diffusely distributed in the brain parenchyma, roughly correlating with areas of viral infection. In the cerebrospinal fluid (CSF) of such animals, high titers of interleukin 8 are found (Tipold *et al.*, 1999). It has been suggested that initial macrophage/microglial cell activation, which occurs in distemper (Alldinger *et al.*, 1996), may trigger invasion of T cells into the CNS (Tipold *et al.*, 1999). Antiviral cytotoxic immune reactions have been shown only in the later stage of the disease (Appel *et al.*, 1982). It is uncertain, therefore, whether the invading cells have any effect on infected cells.

The chronic stage of distemper: immunopathological complications

Coinciding with the recovery of the immune system, perivascular cuffing with lymphocytes, plasma cells and

monocytes occurs in the initial virus-induced brain lesions (Vandevelde *et al.*, 1981). The inflammatory reaction in the demyelinating lesions can lead to progression of the tissue damage (Wisniewski *et al.*, 1972; Vandevelde *et al.*, 1982b). There is often necrosis of the tissue in such lesions, even though a significant number of oligodendrocytes is still present in chronic, completely demyelinated lesions (Schobesberger *et al.*, 2002). Thus, the chronic stage of the disease is mainly characterized by immunopathological complications.

It has been known for many years that anti-myelin antibodies in serum occur in distemper (Krakowka *et al.*, 1973). We found such antibodies also in the CSF of dogs with distemper, and that these antibodies are produced locally in the inflammatory brain lesions (Vandevelde *et al.*, 1986). A cell-mediated response against myelin basic protein was found in four of 11 dogs experimentally infected with CDV (Cerruti Sola *et al.*, 1983). However, neither anti-myelin antibodies nor cell-mediated anti-myelin reactions correlate with the course of the disease. In addition, distemper has no resemblance to experimental autoimmune encephalitis (Summers *et al.*, 1984b).

The initial intrathecal immune response in distemper during the immunosuppressive stage of the disease consists in diffuse invasion of $CD8^+$ T cells (Tipold *et al.*, 1999). During immune recovery, a mature immune reaction develops by perivascular infiltration of $CD4^+$ cells and subsequent recruitment of large numbers of plasma cells and elevated antibody synthesis (Vandevelde *et al.*, 1981, 1986; Tipold *et al.*, 1999; Wunschmann *et al.*, 1999). The titers of CDV-neutralizing antibodies in the CSF often exceed those in the serum (Bollo *et al.*, 1986). Binding studies show that antibodies are made against all proteins of CDV (Johnson *et al.*, 1988). The occurrence of anti-CDV antibodies in the CSF coincided with clearance of CDV and CDV-containing cells from the inflammatory lesions (Bollo *et al.*, 1986; Baumgärtner *et al.*, 1989; Alldinger *et al.*, 1993b).

We found that antiviral antibodies bound to the surface of CDV-infected cells interacted with the Fc receptors of neighbouring macrophages by way of their Fc portions (Bürge *et al.*, 1989; Griot *et al.*, 1989a, b). This interaction resulted in a respiratory burst in macrophages, with release of reactive oxygen species (ROS). We could also show that stimulation of macrophages by way of their Fc receptors or other means, including antiviral antibody–virus immune complexes, led to selective destruction of oligodendrocytes in their vicinity (Griot *et al.*, 1990; Griot-Wenk *et al.*, 1991; Botteron *et al.*, 1992). These experiments showed how the humoral antiviral immune response could lead to destruction of oligodendrocytes as innocent bystander cells (Griot *et al.*, 1990). Obviously, several products secreted by stimulated macrophages/microglia, including ROS, can be made responsible for damage to the oligodendrocyte/myelin compartment. ROS produced chemically using the xanthine/xanthine oxidase system, selectively damaged cultured oligodendrocytes when added to the culture supernatant (Griot *et al.*, 1990); these cells are rich in transferrin and therefore contain a considerable iron load (Griot and Vandevelde, 1988), rendering them particularly vulnerable to ROS attacks. The procoagulant activity of macrophages was markedly enhanced after CDV infection (Brügger *et al.*, 1992), which may enhance the destructive potential of macrophages and provide further support for the hypothesis that bystander demyelination occurs in chronic distemper. The experimental conditions in the antibody experiments *in vitro* closely mimic the situation *in vivo*, in which CDV-infected glial cells in the white matter are in close contact with macrophages/microglia and antiviral antibody-producing cells. Therefore, it is not unreasonable to conclude that a bystander mechanism associated with the antiviral immune response is responsible for the progression of demyelinating lesions in the chronic stage of CDV infection (Griot *et al.*, 1990).

Further progression of the disease: virus persistence

The antiviral immune response should be beneficial to the host insofar as CDV is removed from the tissue (Bollo *et al.*, 1986). However, our studies also showed that CDV can persist in white matter areas outside the inflammatory demyelinating lesions (Bollo *et al.*, 1986). It appears, therefore, that a chronic progressive disease develops if the intrathecal immune response keeps lagging behind viral replication. Thus, viral persistence must be the key to the pathogenesis of the chronic lesions. We found that persistent CDV spreads in a non-cytolytic manner by way of cell processes with very limited budding and release of infectious virus (Zurbriggen *et al.*, 1995) compared with attenuated viruses. In addition, CDV is capable of producing restricted infection in neurons without expressing viral protein (Müller *et al.*, 1995). Others have found restricted expression of surface proteins in the CNS (Alldinger *et al.*, 1993a). Persistence of CDV appears to be related to mechanism(s) preventing recognition of the virus by immunocompetent cells. As sequencing studies have shown differences between virulent and attenuated CDV at the level of the NP, M, F and H genes (Stettler *et al.*, 1997; Cherpillod *et al.*, 1999), it will be difficult to relate persistence to single molecular determinants.

Prevention of CDV infection

Much has been learned about the development of the CNS lesions in canine distemper. Some of these findings may provide a basis for the development of therapeutic strategies, in particular at the immunomodulatory level and/or in reactivating oligodendrocytes from a progeni-

tor pool, even in chronically demyelinated lesions (Schobesberger *et al.*, 2002). However, while modification of the detrimental inflammatory response may become feasible, the basic problem remains the presence of the virus in the CNS.

Effective antiviral therapies against morbilliviruses are not available yet. Therefore, the most important veterinary intervention remains prevention. Whereas vaccines against CDV have been available for a long time and have greatly reduced the incidence of the disease, avianized strains offer inadequate protection against the nervous form of CDV (Tipold *et al.*, 1996), and other vaccine strains may cause postvaccinal encephalitis (Hartley, 1974; Cornwell *et al.*, 1988). However, it is most important to have significant information on disease pathogenesis before any therapeutic or preventive approach is attempted.

It might be impossible to eradicate CDV because of its global distribution and the wide variety of susceptible wildlife species, which include marine and freshwater seals, African cats and many others. Therefore, on the basis of solid information on the pathogenesis of CDV, the development of new preventive approaches, such as the use of recombinant DNA vaccines, has been undertaken. Recently, a DNA vaccine derived from virulent CDV sequences was developed (Cherpillod *et al.*, 2000). Plasmids were constructed containing the nucleocapsid, fusion protein and attachment protein genes of virulent CDV. Preliminary studies have shown that this vaccine induces both humoral and cellular immune response against CDV antigens, and protects from virulent CDV challenge (Cherpillod *et al.*, 2000). Furthermore, the immune response in puppies has been evaluated after priming vaccination with CDV DNA followed by conventional vaccination. The combination of the two vaccines induced a strong humoral immune response (unpublished results, C. Griot and A. Zurbriggen).

Conclusions

Novel morbilliviruses have emerged in recent years, either as newly identified viruses or as viruses crossing species barriers. The observation of morbilliviruses causing re-emerging and newly emerging diseases is consistent with the fact that RNA viruses have a considerable potential to mutate, and therefore can easily adapt to new hosts within a short period (Domingo and Holland, 1994; Cleaveland *et al.*, 2001). CDV has been in the carnivore population for many years and molecular epidemiological evidence suggests that the virus has not changed in the past.

Canine distemper is a well-studied member of the *Morbillivirus* genus, and investigation of the pathogenesis of CDV infection might provide information as to the pathogenesis of related viruses within the same family. New morbillivirus-like viruses have shown zoonotic potential and therefore are of relevance for public health. There is a need for better preventive and diagnostic tools in the future because the vectors carrying some of these viruses are also present in wildlife and may hardly ever be eradicated (Daszak *et al.*, 2000).

Thus, morbilliviruses continue to surprise us. With new molecular techniques at hand and our present knowledge of the disease mechanism of prototype members of the Paramyxoviridae, we are in a better position to rapidly identify, understand and design preventive or even therapeutic measures against them.

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References

Alldinger S, Baumgärtner W and Örvell C (1993a). Restricted expression of viral surface proteins in canine distemper encephalitis. *Acta Neuropathologica (Berlin)* **85**: 635–645.

Alldinger S, Baumgärtner W, van Moll P and Örvell C (1993b). In vivo and in vitro expression of canine distemper viral proteins in dogs and non-domestic carnivores. *Archives of Virology* **132**: 421–428.

Alldinger S, Wunschmann A, Baumgärtner W, Voss C and Kremmer E (1996). Up-regulation of major histocompatibility complex class II antigen expression in the central nervous system of dogs with spontaneous canine distemper virus encephalitis. *Acta Neuropathologica (Berlin)* **92**: 273–280.

Appel MJ (1969). Pathogenesis of canine distemper. *American Journal of Veterinary Research* **30**: 1167–1182.

Appel MJ, Glickman LT, Raine CS and Tourtellotte WW (1981). Canine viruses and multiple sclerosis. *Neurology* **31**: 944–949.

Appel MJ, Shek WR and Summers BA (1982). Lymphocyte-mediated immune cytotoxicity in dogs infected with virulent canine distemper virus. *Infection and Immunity* **37**: 592–600.

Appel MJG, Yates RA and Foley GL (1994). Canine distemper epizootic in lions, tigers and leopards in North America. *Journal of Veterinary Diagnostic Investigation* **6**: 277–288.

Barben G, Stettler M, Jaggy A, Vandevelde M and Zurbriggen A (1999). Detection of IgM antibodies against the nucleocapsid protein of canine distemper virus in dog sera using a dot-blot-assay. *Journal of Veterinary Medicine A* **46**: 115–121.

Barclay AJ and Paton DJ (2000). Hendra (equine morbillivirus). *Veterinary Journal* **160**: 169–176.

Barrett T (1999). Morbillivirus infections, with special emphasis on morbilliviruses of carnivores. *Veterinary Microbiology* **69**: 3–13.

Barrett T, Visser IKG, Mamaev L, Goatley L, Van Bressem MF and Osterhaus ADME (1993). Dolphin and porpoise morbilliviruses are genetically distinct from phocine distemper virus. *Virology* **193**: 1010–1012.

Baumgärtner W, Örvell C and Reinacher M (1989). Naturally occurring canine distemper virus encephalitis: distribution and expression of viral polypeptides in nervous tissues. *Acta Neuropathologica* **78**: 504–512.

Blakemore WF, Summers BA and Appel MG (1989). Evidence of oligodendrocyte infection and degeneration in canine distemper encephalomyelitis. *Acta Neuropathologica* **77**: 550–553.

Bollo E, Zurbriggen A, Vandevelde M and Fankhauser R (1986). Canine distemper virus clearance in chronic inflammatory demyelination. *Acta Neuropathologica* **72**: 69–73.

Bolt G, Jensen TD, Gottschalck E, Arctander P, Appel MJ, Buckland R and Blixenkrone-Moller M (1997). Genetic diversity of the attachment (H) protein gene of current field isolates of canine distemper virus. *Journal of General Virology* **78**: 367–372.

Botteron C, Zurbriggen A, Griot C and Vandevelde M (1992). Canine distemper virus-immune complexes induce bystander degeneration of oligodendrocytes. *Acta Neuropathologica* **83**: 402–407.

Brown C (1997). Emerging diseases—what veterinarians need to know. *Journal of Veterinary Diagnostic Investigation* **9**: 113–117.

Brügger M, Jungi TW, Zurbriggen A and Vandevelde M (1992). Canine distemper virus increases procoagulant activity of macrophages. *Virology* **190**: 616–623.

Bürge T, Griot C, Vandevelde M and Peterhans E (1989). Antiviral antibodies stimulate production of reactive oxygen species in cultured canine brain cells infected with canine distemper virus. *Journal of Virology* **63**: 2790–2797.

Bush M, Montali RJ, Brownstein D, James AE Jr and Appel MJ (1976). Vaccine-induced canine distemper in a lesser panda. *Journal of the American Veterinary Medical Association* **169**: 959–960.

Carpenter JW, Appel MJ, Erickson RC and Novilla MN (1976). Fatal vaccine-induced canine distemper virus infection in black-footed ferrets. *Journal of the American Veterinary Medical Association* **169**: 961–964.

Cerruti Sola S, Kristensen F, Vandevelde M, Bichsel P and Kihm U (1983). Lymphocyte responsiveness to lectin and myelin antigens in canine distemper infection in relation to the development of demyelinating lesions. *Journal of Neuroimmunology* **4**: 77–90.

Cherpillod P, Beck K, Zurbriggen A and Wittek R (1999). Sequence analysis and expression of the attachment and fusion proteins of canine distemper virus wild-type strain A75/17. *Journal of Virology* **73**: 2263–2269.

Cherpillod P, Tipold A, Griot-Wenk M, Cardozo C, Schmid I, Fatzer R, Schobesberger M, Zurbriggen R, Bruckner L, Roch F, Vandevelde M, Wittek R and Zurbriggen A (2000). DNA encoding nucleocapsid and surface proteins of wild type canine distemper virus protects its natural host against distemper. *Vaccine* **18**: 2927–2936.

Chua KB, Goh KJ, Wong KT, Kamarulzaman A, Tan PS, Ksiazek TG, Zaki SR, Paul G, Lam SK and Tan CT (1999). Fatal encephalitis due to Nipah virus among pig-farmers in Malaysia. *Lancet* **354**: 1256–1259.

Chua KB, Bellini WJ, Rota PA, Harcourt BH, Tamin A, Lam SK, Ksiazek TG, Rollin PE, Zaki SR, Sheih WJ, Goldsmith CS, Gubler DJ, Roehrig JT, Eaton B, Gould AR, Olson J, Field H, Daniels P, Ling AE, Peters CJ, Anderson LJ and Mahy WJ (2000). Nipah virus: a recently emergent deadly paramyxovirus. *Science* **288**: 1432–1435.

Chua KB, Koh CL, Hooi PS, Wee KF, Khong JH, Chua BH, Chan YP, Lim ME and Lam SK (2002). Isolation of Nipah virus from Malaysian island flying-foxes. *Microbes and Infection* **4**: 145–151.

Cleaveland S, Appel MG, Chalmers WS, Chillingworth C, Kaare M and Dye C (2000). Serological and demographic evidence for domestic dogs as a source of canine distemper virus infection for Serengeti wildlife. *Veterinary Microbiology* **72**: 217–227.

Cleaveland S, Laurenson MK and Taylor LH (2001). Diseases of humans and their domestic mammals: pathogen characteristics, host range and the risk of emergence. *Philosophical Transactions of the Royal Society of London Series B, Biological Sciences* **356**: 991–999.

Cook SD, Dowling PC and Russell WC (1979). Neutralizing antibodies to canine distemper and measles virus in multiple sclerosis. *Journal of the Neurological Sciences* **41**: 61–70.

Cook SD and Dowling PC (1980). Multiple sclerosis and viruses: an overview. *Neurology* **30**: 80–91.

Cook SD, Gudmundsson G, Benedikz J and Dowling PC (1980). Multiple sclerosis and distemper in Iceland 1966–1978. *Acta Neurologica Scandinavica* **61**: 244–251.

Cook SD, Blumberg B and Dowling PC (1986). Potential role of paramyxoviruses in multiple sclerosis. *Neurologic Clinics* **4**: 303–319.

Cook SD, Rohowsky-Kochan C, Bansil S and Dowling PC (1995). Evidence for multiple sclerosis as an infectious disease. *Acta Neurologica Scandinavica Supplement* **161**: 34–42.

Cornwell HJ, Thompson H, McCandlish IA, Macartney L and Nash AS (1988). Encephalitis in dogs associated with a batch of canine distemper (Rockborn) vaccine. *Veterinary Record* **122**: 54–59.

Dal Canto MC and Rabinowitz SG (1982). Experimental models of virus-induced demyelination of the central nervous system. *Annals of Neurology* **11**: 109–127.

Daszak P, Cunningham AA and Hyatt AD (2000). Emerging infectious diseases of wildlife-threats to biodiversity and human health. *Science* **287**: 443–449.

Deem SL, Spelman, LH, Yates RA and Montali RJ (2000). Canine distemper in terrestrial carnivores: a review. *Journal of Zoo and Wildlife Medicine* **31**: 441–451.

De Keyser J, Wilczak N, Walter JH and Zurbriggen A (2001). Disappearance of beta2-adrenergic receptors on astrocytes in canine distemper encephalitis: possible implications for the pathogenesis of multiple sclerosis. *Neuroreport* **12**: 191–194.

Domingo E and Holland JJ (1994). Mutation rates and rapid evolution of RNA viruses. In: Morse SS (editor). *The Evolutionary Biology of Viruses*. New York: Raven Press, pp. 151–184.

Duignan PJ, Saliki JT, St Aubin DJ, Early G, Sadove S, House JA, Kovacs K and Geraci JR (1995). Epizootiology of morbillivirus infection in North American harbor seals (*Phoca vitulina*) and gray seals (*Halichoerus grypus*). *Journal of Wildlife Diseases* **31**: 491–501.

Ek-Kommonen C, Sihvonen L, Pekkanen K, Rikula U and Nuotio L (1997). Outbreak of canine distemper in vaccinated dogs in Finland. *Veterinary Record* **141**: 380–383.

Gemma T, Watari T, Akiyama K, Miyashita N, Shin YS, Iwatsuki K, Kai C and Mikami T (1996). Epidemiological observations on recent outbreaks of canine distemper in Tokyo area. *Journal of Veterinary Medical Science* **58**: 547–550.

Gilden DH (2002). A search for virus in multiple sclerosis. *Hybridoma and Hybridomics* **21**: 93–97.

Glaus T, Griot C, Richard A, Althaus U, Herschkowitz N and Vandevelde M (1990). Ultrastructural and biochemical findings in brain cell cultures infected with canine distemper virus. *Acta Neuropathologica* (*Berlin*) **80**: 59–67.

Graber H, Müller C, Vandevelde M and Zurbriggen A (1995).

Restricted infection with canine distemper virus leads to down-regulation of myelin gene transcription in cultured oligodendrocytes. *Acta Neuropathologica (Berlin)* **90**: 312–318.

Griot C and Vandevelde M (1988). Transferrin, carbonic anhydrase C and ferritin in dissociated murine brain cell cultures. *Journal of Neuroimmunology* **18**: 333–340.

Griot C, Bürge T, Vandevelde M and Peterhans E (1989a). Antibody-induced generation of reactive oxygen radicals by brain macrophages in canine distemper encephalitis: a mechanism for bystander demyelination. *Acta Neuropathologica* **78**: 396–403.

Griot C, Bürge T, Vandevelde M and Peterhans E (1989b). Bystander demyelination through antibody induced macrophage activation in canine distemper virus infection. *Schweizer Archiv für Neurologie, Neurochirurgie und Psychiatrie* **140**: 39–41.

Griot C, Vandevelde M, Richard A, Peterhans E and Stocker R (1990). Selective degeneration of oligodendrocytes mediated by reactive oxygen species. *Free Radical Research Communications* **11**: 181–193.

Griot-Wenk M, Griot C, Pfister H and Vandevelde M (1991). Antibody-dependent cellular cytotoxicity in anti-myelin antibody-induced oligodendrocyte damage in vitro. *Journal of Neuroimmunology* **33**: 145–155.

Harcourt BH, Tamin A, Ksiazek TG, Rollin PE, Anderson LJ, Bellini WJ and Rota PA (2000). Molecular characterization of Nipah virus, a newly emergent paramyxovirus. *Virology* **271**: 334–349.

Harder TC, Kenter M, Appel MJG, Roelke-Parker ME, Barrett T and Osterhaus ADME (1995). Phylogenetic evidence of canine distemper virus in Serengeti's lions. *Vaccine* **13**: 521–523.

Hartley WJ. (1974). A post-vaccinal inclusion body encephalitis in dogs. *Veterinary Pathology* **11**: 301–312.

Higgins RJ, Krakowka SG, Metzler A and Koestner A (1982). Primary demyelination in experimental canine distemper virus induced encephalomyelitis in gnotobiotic dogs. Sequential immunologic and morphologic findings. *Acta Neuropathologica* **58**: 1–8.

Hooper PT and Williamson MM (2000). Hendra and Nipah virus infections. *Emerging Infectious Diseases* **16**: 597–603.

Hughes RA, Russell WC, Froude JR and Jarrett RJ (1980). Pet ownership, distemper antibodies and multiple sclerosis. *Journal of the Neurological Sciences* **47**: 429–432.

Ikeda Y, Nakamura K, Miyazawa T, Chen MC, Kuo TF, Lin JA, Mikami T, Kai C and Takahashi E (2001). Seroprevalence of canine distemper virus in cats. *Clinical and Diagnostic Laboratory Immunology* **8**: 641–644.

Jensen T, van de Bildt M, Dietz HH, Andersen TH, Hammer AS, Kuiken T, Osterhaus A (2002). Another phocine distemper outbreak in Europe. *Science* **297**: 209.

Iwatsuki K, Miyashita N, Yoshida E, Gemma T, Shin YS, Mori T, Hirayama N, Kai C and Mikami T (1997). Molecular and phylogenetic analyses of the haemagglutinin (H) proteins of field isolates of canine distemper virus from naturally infected dogs. *Journal of General Virology* **78**: 373–380.

Johnson GC, Fenner WR and Krakowka S (1988). Production of immunoglobulin G and increased antiviral antibody in cerebrospinal fluid of dogs with delayed-onset canine distemper viral encephalitis. *Journal of Neuroimmunology* **17**: 237–251.

Johnson R, Glickman LT, Emerick TJ and Patronek GJ (1995). Canine distemper infection in pet dogs: I. Surveillance in Indiana during a suspected outbreak. *Journal of the American Animal Hospital Association* **31**: 223–229.

Kennedy S, Kuiken T, Jepson PD, Deaville R, Forsyth M, Barrett T, van de Bildt MWG, Osterhaus ADME, Eybatov T, Duck C, Kydyrmanov A, Mitrofanov I and Wilson S (2000). Mass die-off of Caspian seals caused by canine distemper virus. *Emerging Infectious Diseases* **6**: 637–639.

Krakowka S (1982). Mechanisms of in vitro immunosuppression in canine distemper virus infection. *Journal of Clinical and Laboratory Immunology* **8**: 187–196.

Krakowka S and Koestner A (1978). Canine distemper virus and multiple sclerosis. *Lancet* **1**: 1127–1128.

Krakowka S, McCullough B, Koestner A and Olsen R (1973). Myelin-specific auto-antibodies associated with central nervous system demyelination in canine distemper virus infection. *Infection and Immunity* **8**: 819–827.

Krakowka S, Higgins RJ and Koestner A (1980). Canine distemper virus: review of structural and functional modulations in lymphoid tissues. *American Journal of Veterinary Research* **41**: 284–292.

Lam SK and Chua KB (2002). Nipah virus encephalitis outbreak in Malaysia. *Clinical Infectious Diseases* **34**: 48–51.

Leisewitz AL, Carter A, van Vuuren M and van Blerk L (2001). Canine distemper infections, with special reference to South Africa, with a review of the literature. *Journal of the South African Veterinary Association* **72**: 127–136.

Mackenzie JS, Chua KB, Daniels PW, Eaton BT, Field HE, Hall RA, Pin K, Johansen CA, Kirkland PD, Lam SK, McMinn P, Nisbet DJ, Paru R, Pyke AT, Ritchie SA, Siba P, Smith DW, Smith GA, van den Hurk AF, Wang LF and Williams DT (2001). Emerging viral diseases of Southeast Asia and the Western Pacific. *Emerging Infectious Diseases* **7**: 497–504.

Madden DL, Wallen WC, Houff SA, Shekarchi IC, Leinikki PO, Castellano GA and Sever JL (1981). Measles and canine distemper antibody. Presence in sera from patients with multiple sclerosis and matched control subjects. *Archives of Neurology* **38**: 13–15.

Mayo MA (2002). Virus taxonomy—Houston 2002. *Archives of Virology* **147**: 1071–1076.

McCullough B, Krakowka S and Koestner A (1974). Experimental canine distemper virus-induced demyelination. *Laboratory Investigation* **31**: 216–222.

Morell V (1996). New virus variant killed Serengeti cats. *Science* **271**: 596.

Müller C, Fatzer R, Beck K, Vandevelde M and Zurbriggen A (1995). Studies on canine distemper virus persistence in the nervous system. *Acta Neuropathologica (Berlin)* **89**: 438–445.

Murray PK, Selleck P, Hooper P, Hyatt A, Gould A, Gleeson L, Westbury H, Hiley L, Selvey L, Rodwell B and Ketterer P (1995). A morbillivirus that caused fatal disease in horses and humans. *Science* **268**: 94–97.

Murray PK, Eaton B, Hooper P, Wang L, Williamson M and Young P (1998). Flying foxes, horses and humans: a zoonosis caused by a new member of the Paramyxoviridae. In: Scheld WM, Armstrong O and Hughes JM (editors). *Emerging Infections 1*. Washington, DC: ASM Press, pp. 43–58.

Mutinelli F, Vandevelde M, Griot C and Richard A (1989). Astrocytic infection in canine distemper virus-induced demyelination. *Acta Neuropathologica (Berlin)* **77**: 333–335.

Olson JG, Rupprecht C, Rollin PE, An US, Niezgoda M, Clemins T, Walston J and Ksiazek TG (2002). Antibodies to Nipah-like virus in bats (Pteropus lylei), Cambodia. *Emerging Infectious Diseases* **8**: 987–988.

Osterhaus ADME (2001). Catastrophes after crossing species barriers. *Philosophical Transactions of the Royal Society of London Series B Biological Sciences* **356**: 791–793.

Osterhaus ADME and Vedder EJ (1988). Identification of virus causing recent seal deaths. *Nature* **335**: 20.

Osterhaus ADME, Groen J, De Vries P, Uytdehaag FGCM, Klingeborn B and Zarnke R (1988). Canine distemper virus in seals [letter]. *Nature* **335**: 403–404.

Osterhaus ADME, Groen J, UytdeHaag FGCM, Visser IKG, van de Bildt MWG, Bergman A and Klingeborn B (1989). Distemper virus in Baikal seals. *Nature* **338**: 209–210.

O'Sullivan JD, Allworth AM, Paterson DL, Snow TM, Boots R, Gleeson LJ, Gould AR, Hyatt AD and Bradfield J (1997). Fatal encephalitis due to novel paramyxovirus transmitted from horses. *Lancet* **349**: 93–95.

Raine CS (1976). On the development of CNS lesions in natural canine distemper encephalomyelitis. *Journal of the Neurological Sciences* **30**: 13–28.

Roelke-Parker ME, Munson L, Packer C, Kock R, Cleaveland S, Carpenter M, O'Brien SJ, Pospischil A, Hofmann-Lehmann R, Lutz H, Mwamengele GLM, Mgasa MN, Machange GA, Summers BA and Appel MJG (1996). A canine distemper virus epidemic in Serengeti lions (Panthera leo). *Nature* **379**: 441–445.

Rogers RJJ, Douglas IC, Baldock FC, Glanville RJ, Seppanen KT, Gleeson LJ, Selleck PN and Dunn KJ (1996). Investigation of a second focus of equine morbillivirus infection in coastal Queensland. *Australian Veterinary Journal* **74**: 243–244.

Rohowsky-Kochan C, Dowling PC and Cook SD (1995). Canine distemper virus-specific antibodies in multiple sclerosis. *Neurology* **45**: 1554–1560.

Schobesberger M, Zurbriggen A, Summerfield A, Vandevelde M and Griot C (1999). Oligodendroglial degeneration in distemper: apoptosis or necrosis? *Acta Neuropathologica* (*Berlin*) **97**: 179–187.

Schobesberger M, Zurbriggen A, Doherr MG, Weissenböck H, Vandevelde M, Lassmann H and Griot C (2002). Demyelination precedes oligodendrocyte loss in canine distemper virus-induced encephalitis. *Acta Neuropathologica* (*Berlin*) **103**: 11–19.

Stettler M and Zurbriggen A (1995). Nucleotide and deduced amino acid sequences of the nucleocapsid protein of the virulent A75/17-CDV strain of canine distemper virus. *Veterinary Microbiology* **44**: 211–217.

Stettler M, Beck K, Wagner A, Vandevelde M and Zurbriggen A (1997). Determinants of persistence in canine distemper viruses. *Veterinary Microbiology* **57**: 83–89.

Summers BA and Appel MJ (1987). Demyelination in canine distemper encephalomyelitis: an ultrastructural analysis. *Journal of Neurocytology* **16**: 871–881.

Summers BA, Greisen HA and Appel MJ (1979). Early events in canine distemper demyelinating encephalomyelitis. *Acta Neuropathologica* (*Berlin*) **46**: 1–10.

Summers BA, Greisen HA and Appel MJ (1984a). Canine distemper encephalomyelitis: variation with virus strain. *Journal of Comparative Pathology* **94**: 65–75.

Summers BA, Greisen HA and Appel MJ (1984b). Canine distemper and experimental allergic encephalomyelitis in the dog: comparative patterns of demyelination. *Journal of Comparative Pathology* **94**: 575–589.

Summers BA, Cummings JF and deLahunta A (1995). Canine distemper. In: *Veterinary Neuropathology*. St Louis: Mosby, pp. 102–110.

Tipold A, Jaggy A, Zurbriggen A and Vandevelde M (1996). Neurological signs in canine distemper encephalomyelitis—a clinical study. *European Journal of Companion Animal Practice* **6**: 33–38.

Tipold A, Moore P, Zurbriggen A, Burgener I, Barben G and Vandevelde M (1999). Early T-cell response in the central nervous system in canine distemper virus infection. *Acta Neuropathologica* (*Berlin*) **97**: 45–56.

Van de Bildt MWG, Kuiken T, Visee AM, Lema S, Fitzjohn TR and Osterhaus ADME (2002). Distemper outbreak and its effect on African wild dog conservation. *Emerging Infectious Diseases* **8**: 211–213.

Vandevelde M and Zurbriggen A (1995). The neurobiology of canine distemper virus infection. *Veterinary Microbiology* **44**: 271–280.

Vandevelde M, Fankhauser R, Kristensen F and Kristensen B (1981). Immunoglobulins in demyelinating lesions in canine distemper encephalitis. An immunohistological study. *Acta Neuropathologica* (*Berlin*) **54**: 31–41.

Vandevelde M, Higgins RJ, Kristensen B, Kristensen F, Steck AJ and Kihm U (1982a). Demyelination in experimental canine distemper virus infection: immunological, pathologic, and immunohistological studies. *Acta Neuropathologica* (*Berlin*) **56**: 285–293.

Vandevelde M, Kristensen F, Kristensen B, Steck AJ and Kihm U (1982b). Immunological and pathological findings in demyelinating encephalitis associated with canine distemper virus infection. *Acta Neuropathologica* (*Berlin*) **56**: 1–8.

Vandevelde M, Zurbriggen A, Dumas M and Palmer D (1985a). Canine distemper virus does not infect oligodendrocytes in vitro. *Journal of the Neurological Sciences* **69**: 133–137.

Vandevelde M, Zurbriggen A, Higgins RJ and Palmer D (1985b). Spread and distribution of viral antigen in nervous canine distemper. *Acta Neuropathologica* (*Berlin*) **67**: 211–218.

Vandevelde M, Zurbriggen A, Steck A and Bichsel P (1986). Studies on the intrathecal humoral immune response in canine distemper encephalitis. *Journal of Neuroimmunology* **11**: 41–51.

Wang LF and Eaton BT (2001). Emerging paramyxoviruses. *Infectious Disease Review* **3**: 52–69.

Wang LF, Yu M, Hansson E, Pritchard LI, Shiell B, Michalski WP and Eaton BT (2000). The exceptionally large genome of Hendra virus: support for creation of a new genus within the family Paramyxoviridae. *Journal of Virology* 74: 9972–9979.

Wisniewski H, Raine CS and Kay WJ (1972). Observations on viral demyelinating encephalomyelitis. Canine distemper. *Laboratory Investigation* **26**: 589–599.

Wunschmann A, Alldinger S, Kremmer E and Baumgärtner W (1999). Identification of $CD4^+$ and $CD8^+$ T cell subsets and B cells in the brain of dogs with spontaneous acute, subacute-, and chronic-demyelinating distemper encephalitis. *Veterinary Immunology and Immunopathology* **67**: 101–116.

Zurbriggen A, Vandevelde M and Dumas M (1986). Secondary degeneration of oligodendrocytes in canine distemper virus infection in vitro. *Laboratory Investigation* **54**: 424–431.

Zurbriggen A, Vandevelde M, Dumas M, Griot C and Bollo E (1987). Oligodendroglial pathology in canine distemper virus infection in vitro. *Acta Neuropathologica* (*Berlin*) **74**: 366–373.

Zurbriggen A, Yamawaki M and Vandevelde M (1993). Restricted canine distemper virus infection of oligodendrocytes. *Laboratory Investigation* **68**: 277–284.

Zurbriggen A, Graber H, Wagner A and Vandevelde M (1995). Canine distemper persistence in the nervous system is associated with non-cytolytic virus spread. *Journal of Virology* **69**: 1678–1686.

Zurbriggen A, Schmid I, Graber HU and Vandevelde M (1998). Oligodendroglial pathology in canine distemper. *Acta Neuropathologica (Berlin)* **95**: 71–77.